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STRUCTURAL POLYPEPTIDES OF HAZARA VIRUS.(U)
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Four structural polypeptides of Hazara virus, an agent closely related to the Crimean-Congo hemorrhagic fever (C-CHF viruses, were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Three glycoproteins were identified (mol. wt. 84,000, 45,000 and 30,000) and found to be associated with the virion envelope. A fourth polypeptide (mol wt. 52,000) was nonglycosylated and associated with the nucleocapsid. The structural proteins of Hazara virus differ markedly from those reported for other bunyaviruses.

Structural Polypeptides of Hazara Virus

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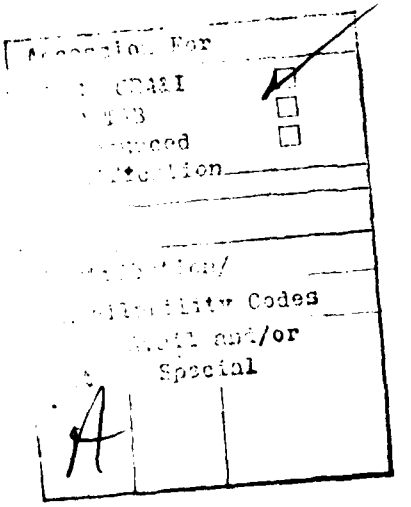

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FOOTNOTES

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SUMMARY

Four structural polypeptides of Hazara virus, an agent closely related to the Crimean-Congo hemorrhagic fever (C-CHF) viruses, were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Three glycoproteins were identified (mol. wt. 84 000, 45 000 and 30 000) and found to be associated with the virion envelope. A fourth polypeptide (mol. wt. 52 000) was nonglycosylated and associated with the nucleocapsid. The structural proteins of Hazara virus differ markedly from those reported for other bunyaviruses.

Hazara virus was isolated in 1964 from ticks collected in the Hazara District of West Pakistan (Begum et al. 1970) and has spurred interest in recent years due to its close serologic relationship with the Crimean-Congo hemorrhagic fever (C-CHF) viruses (Casals & Tignor, 1974). Both Hazara and C-CHF viruses are classified as members of the family Bunyaviridae primarily on the basis of morphologic appearance by electron microscopy (Murphy et al. 1973; Jelínková et al. 1975; Korolev et al. 1976; Smirnova, et al. 1977) and together with others, comprise one of the many unique serogroups within the family (Porterfield et al. 1975/76).

Studies of the molecular structure of these viruses have been stimulated by the desire for an effective vaccine against C-CHF. Efforts have focused on Hazara virus since this agent elicits cross-protection in mice against C-CHF virus challenge, may be safely handled in the laboratory and replicates to ten-fold higher titers in cell culture than C-CHF strains (unpublished observations).

Hazara virus, strain JC280, in the 8th suckling mouse brain (smb) passage was obtained from J. Casals (YARU, New Haven, Conn.), passaged in suckling mice and cloned from the 11th smb passage by three terminal dilution passes in BHK-21 cells. Virus was propagated by inoculation of BHK-21 cell monolayers (5×10^8 cells) with virus (0.1 pfu/cell) and incubation under medium 199 (Earle's) containing 1/40 normal amino acids (GIBCO, Grand Island, N.Y.), 5% dialyzed foetal calf serum (FCS), 0.01 M HEPES buffer and antibiotics. Radiolabeled metabolites (New England Nuclear, Boston, Mass.) were added 4 h postinfection (^{3}H-labelled amino acids, glucosamine or uridine, 10 µCi/ml; ^{14}C-labelled amino acids, 4 µCi/ml) and infected supernatants were harvested 24 h postinfection, centrifuged (380 x g, 10 min) and clarified (8 000 x g, 30 min).

Virus samples were concentrated by direct pelleting (SW27, 116 000 x g, 60 min) or by ammonium sulfate precipitation (Rosato et al. 1974); each procedure yielded similar amounts of virus. Concentrates were resuspended in TNE (0.01 M tris-HCl, 0.1 M NaCl, 0.001 M EDTA) and purified by equilibrium centrifugation (SW50.1, 250 000 x g, 60 min for direct pellets; SW27, 116 000 x g, 4 h for $(NH_4)_2SO_4$ ppt.) on two successive continuous gradients of 20-50% (w/v) sucrose in TNE. Gradient fractions were assayed for radiolabel by liquid scintillation in Scintilute containing 10% (v/v) Scintisol (Isolab, Akron, Ohio) using a Beckman LS8000 beta counter. Infectivity titers were determined by plaque assay with SW-13 cell monolayers (ATCC CCL 105) under medium 199 overlays containing 5% FCS and 0.6% agarose. Plaques were counted following addition of neutral red at three days postinfection.

A representative purification gradient of Hazara virus is shown in Fig. 1a. Infectivity titers closely paralleled a single radioactive peak at a mean density of 1.16 g/cm^3. Purified virus from the appropriate fractions of similar gradients were used to characterize viral components and identify structural proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Purified virus labelled with ^3H-uridine and ^{14}C-amino acids was disrupted at 4°C for 1 h in 2% (v/v) Nonidet P-40 detergent (Shell Oil, Tulsa Okla.); degraded virions were separated into components on 20-60% sucrose gradients (SW27.1, 116 000 x g, 18 h) (Fig. 1b). Three radioactive peaks were observed: a high density (1.26 g/cm^3) uridine-rich peak presumably representing viral nucleocapsid; a small, moderately dense peak (1.16 g/cm^3) probably residual intact virus; and a protein-rich band remaining near the top of the gradient.

Four structural polypeptides of Hazara virus having molecular weights of 84 000, 52 000, 45 000 and 30 000 were resolved using SDS-PAGE as described by Laemmli (1970) (Fig. 2a) from the single peak presented in Fig. 1a. Molecular weights were estimated by co-electrophoresis (3 ma/gel, 2 h) and comparison of ^{14}C-labelled Hazara virus proteins with tritiated Venezuelan equine encephalitis (VEE) and Oriboca virus standards. When virus labelled with ^{3}H-glucosamine and ^{14}C-amino acids was similarly electrophoresed, only one polypeptide species (mol. wt. 52 000) failed to incorporate radioactive glucosamine (Fig. 2b); the three glycoproteins were designated GP1, GP2, and GP3 in order of decreasing molecular weight. The detection of a 45 000 mol. wt. protein generated regarding the true origin of GP2 (cellular or viral), since cellular actin from BHK-21 cells (mol. wt. 43 000) has been copurified with rabies virus (Naito & Matsumoto, 1979). However, cellular actin is nonglycosylated and GP2 incorporated large amounts of radioactive glucosamine.

Gel profiles from the nucleocapsid band and soluble protein fraction of the NP-40-treated sample suggest structural locations of each polypeptide within the virion. The nucleocapsid fraction contained large amounts of the nonglycosylated protein (mol. wt. 52 000); referred to as nucleoprotein, N, and small amounts of GP1 (mol. wt. 84 000). Conversely, soluble fractions from the top of the gradient contained very little N protein while all three glycoproteins were present in approximately normal molar ratios. Extrinsic iodination of intact virus by the glucosoxidase-lactoperoxidase technique (Hubbard & Cohn, 1972) supported these findings in that GP1, GP2, and GP3 were heavily labelled and the N protein was labelled to a much lesser extent (data not shown). Data from both labelling techniques strongly suggest that the virion

envelope is composed of three glycoproteins while the nulceocapsid contains a single, nonglycosylated polypeptide.

The N protein of Hazara virus (mol. wt. 52 000) differs markedly in size from nucleocapsid proteins reported for other bunyaviruses (mol. wt. 19 000-25 000). In addition, the glycoprotein profile is dissimilar to patterns found among other bunyaviruses. However, glycoprotein size varies considerably among Bunyaviridae genera, while nucleocapsid proteins remain in a narrow molecular weight range (Obijeski & Murphy, 1977). Recently, reorganization of the family Bunyaviridae has been proposed to more completely classify this large group of viruses on the basis of serological cross-reactivity and molecular structure. Due to a slight, but detectable, cross-reaction between C-CHF and Nairobi sheep disease viruses (Casals & Tignor, 1980, in press) the C-CHF group has been assigned to the Nairovirus genus in this new system. Polypeptide composition similar to that of Hazara virus has been observed in members of the Nairovirus genus thus far examined (Bishop et al. 1980, in press).

Molecular analysis of C-CHF and related viruses, especially in terms of RNA composition and oligonucleotide fingerprinting, should be actively pursued to facilitate the development of a C-CHF vaccine and to more clearly define the taxonomic status of these viruses.

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FIGURE LEGENDS

Fig. 1. Gradient labelled profiles of purified Hazara virus. (a) Intact virus (pfu/ml, O--O) labelled with ^{3}H-amino acids (●——●) and centrifuged (SW27.1, 116 000 x g, 18 h) in 20-50% (w/v) sucrose in TNE. (b) NP-40 degraded virus labelled with ^{3}H-uridine (●——●) and ^{14}C-labelled amino acids (O--O) and centrifuged (SW27.1, 116 000 x g, 18 h) in 20-60% (w/v) sucrose in TNE.

Fig. 2. Polyacrylamide gels (8%) of Hazara virus, sliced and counted by liquid scintillation. (a) ^{14}C-amino acid labelled Hazara virus (●——●) was mixed with tritiated virus standards (O--O), VEE (mol. wt. 59 000, 53 000, 32 000; Pederson & Eddy, 1975) and Oriboca (mol. wt. 119 000, 32 000, 23 000; Obijeski & Murphy, 1977). VEE and Oriboca virus standards were calibrated using Coomassie brilliant blue stained protein standards (LMW Calibration Kit, Pharmacia Fine Chemicals, Piscataway, N.J.) (Weber & Osborn, 1969). Four polypeptides were resolved and designated GP1, N, GP2 and GP3 in order of decreasing molecular weight. (b) Hazara virus labelled with ^{3}H-glucosamine (●——●) and ^{14}C-amino acids (O--O).

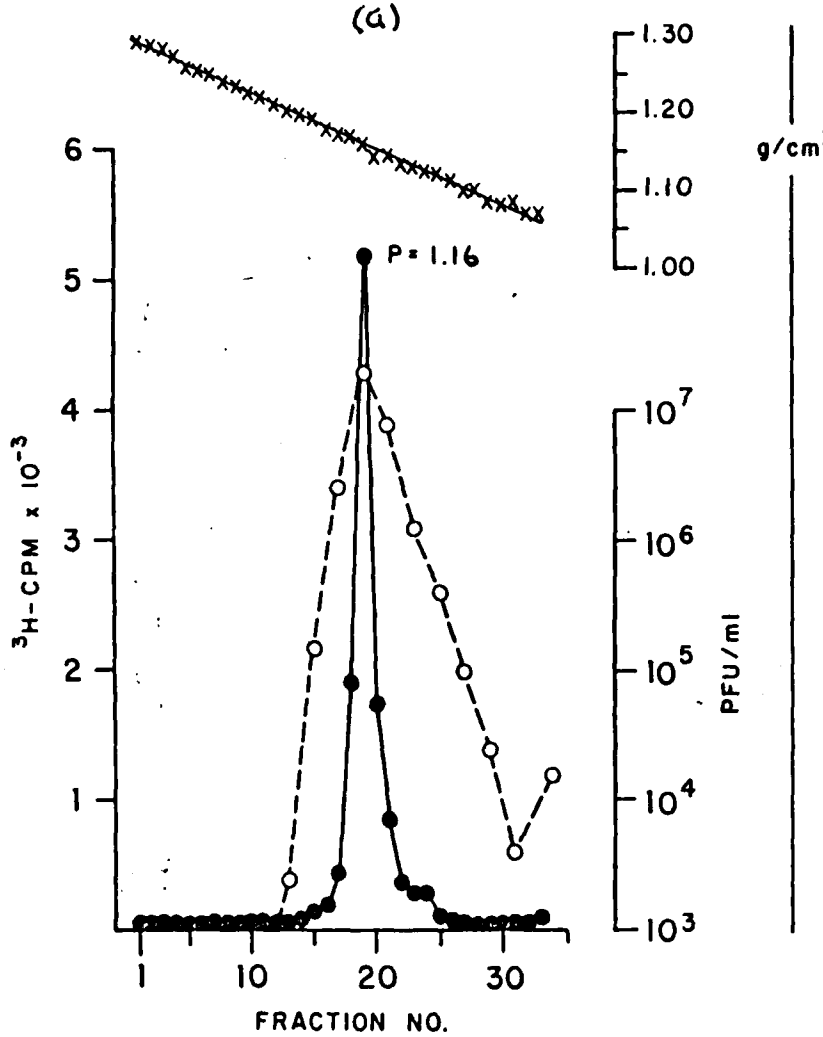

FIG. [redacted] 1a

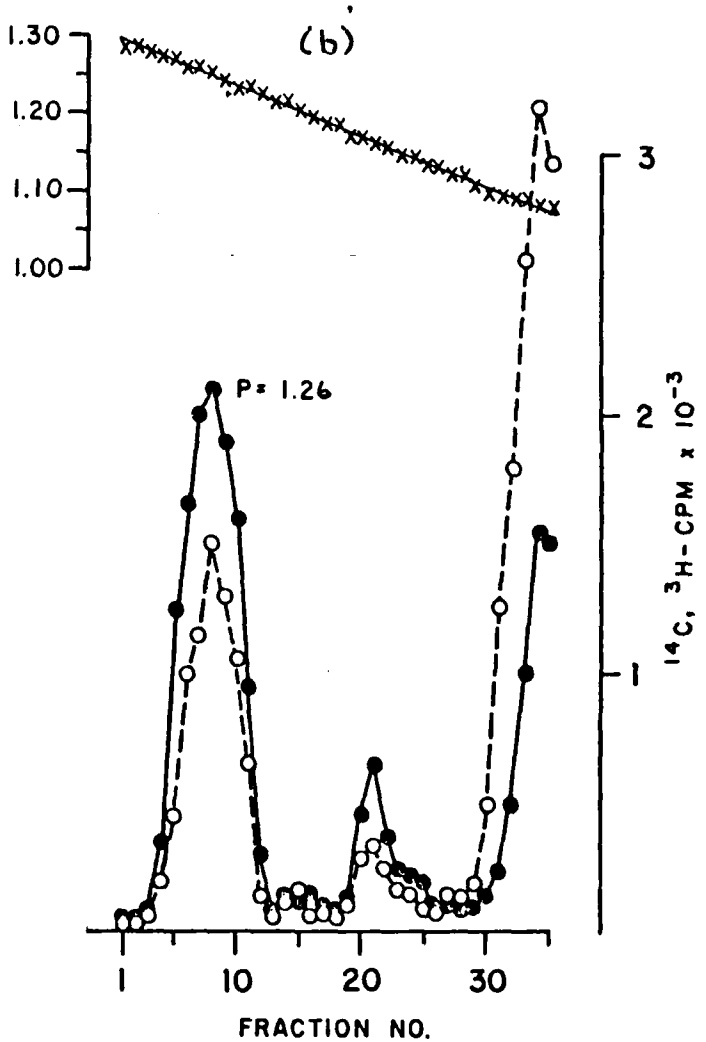

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Fig. 1b

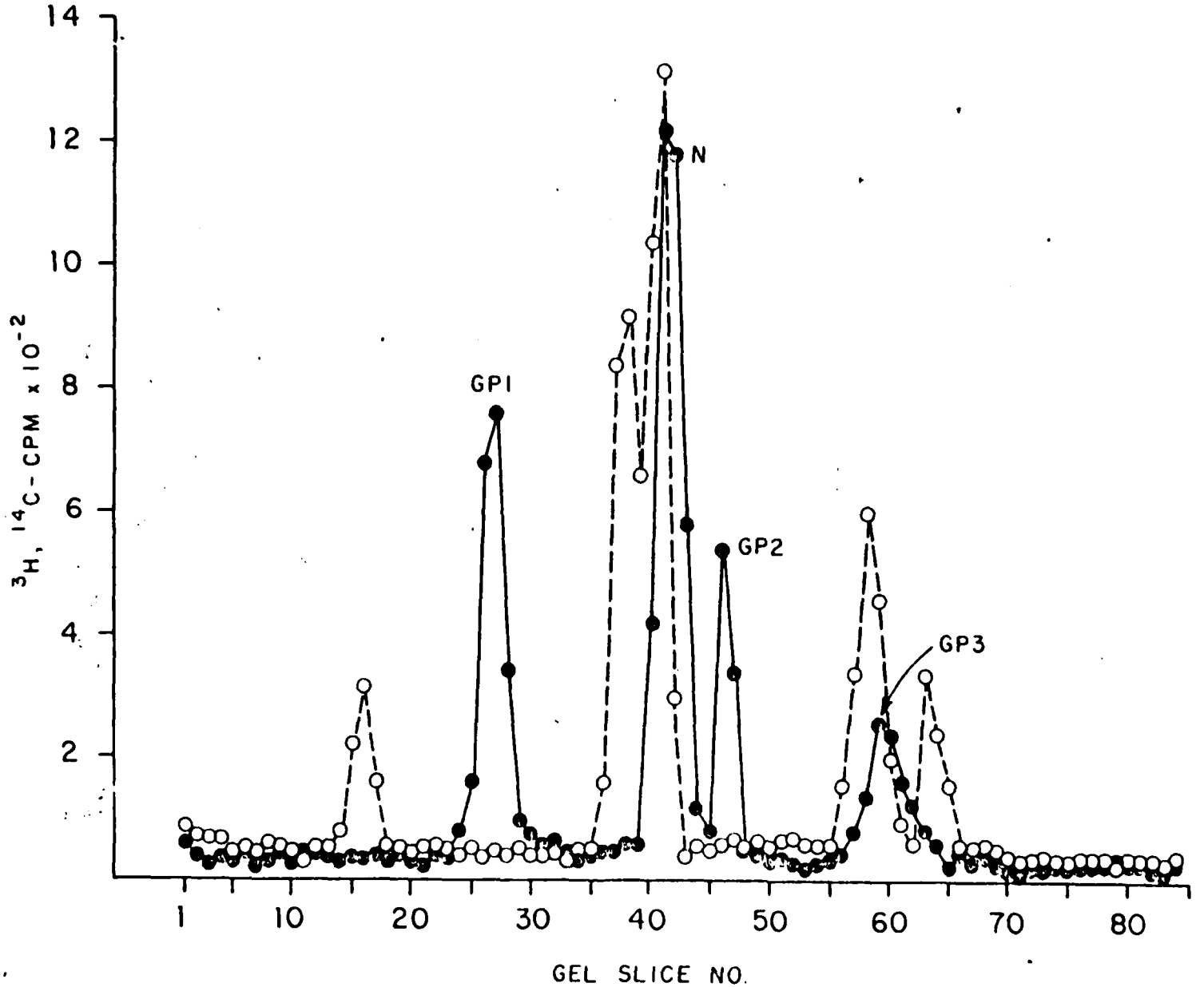
14
12
10
8
6
4
2
^3H, ^{14}C- CPM x 10^{-2}
N
GP1
GP2
GP3
1
10
20
30
40
50
60
70
80
GEL SLICE NO.

Fig. 2a

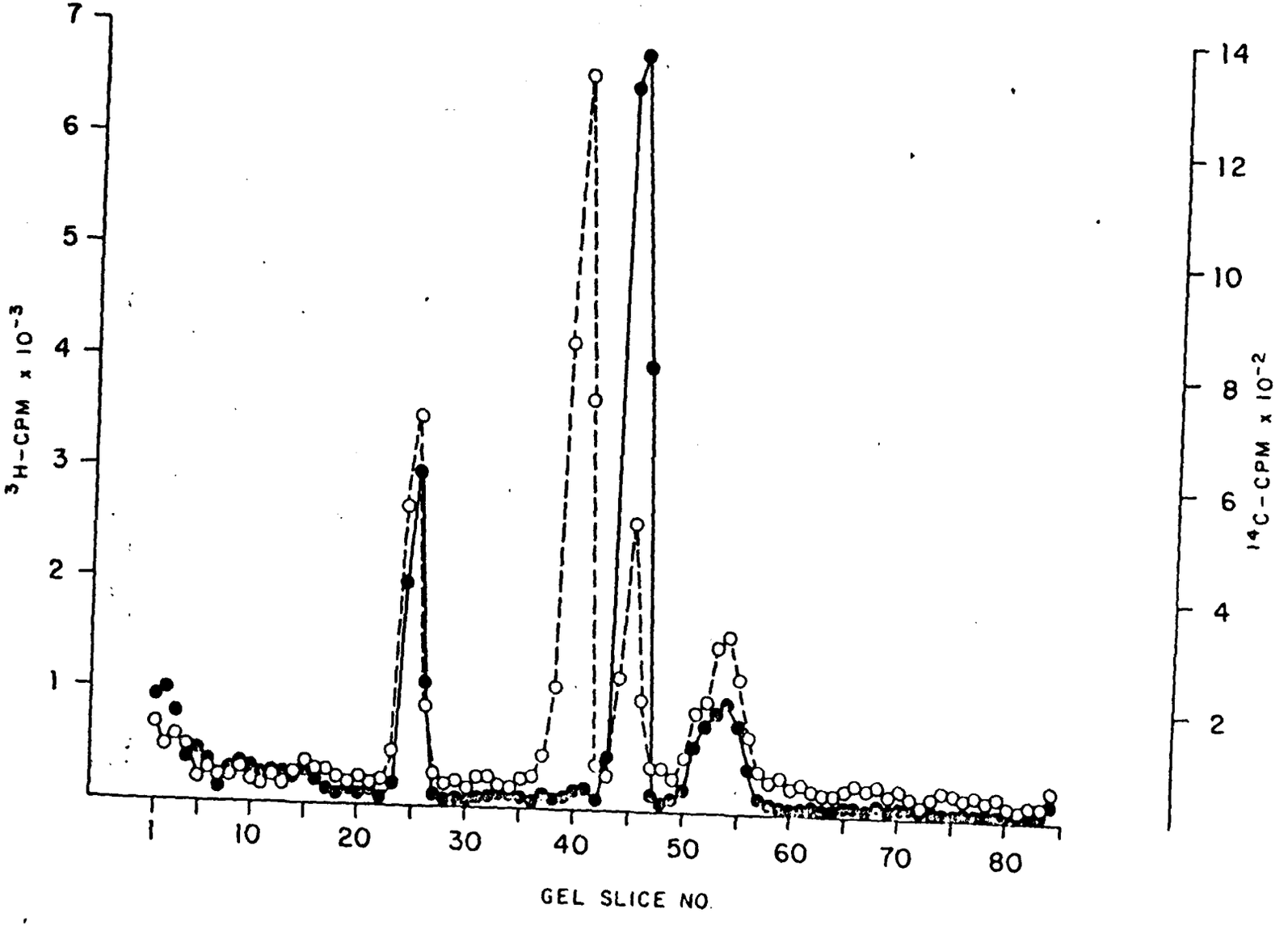
7
6
5
4
3
2
1
^{3}H-CPM x 10^{-3}
14
12
10
8
6
4
2
^{14}C-CPM x 10^{-2}
1
10
20
30
40
50
60
70
80
GEL SLICE NO.

Fig. 2b